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Differentiated effects on splanchnic homeostasis by selective and non-selective endothelin receptor antagonism in porcine endotoxaemia

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1 The non-selective endothelin (ET) receptor antagonist bosentan has been shown to restore systemic and gut oxygen delivery and reverse intestinal mucosal acidosis in porcine endotoxin shock.

2 To further elucidate the specific role of the ET_A as opposed to the ET_B receptor and their effects in the splanchnic region a non-selective (ET_{MIX}ra) A-182086 and selective ET_A (ET_Ara) PD155080 and ET_B (ET_Bra) A-192621 receptor antagonists were administered, separately or simultaneously (ET_{A+B}ra) 2 h after onset of endotoxin shock. These four groups were compared to a control group receiving only endotoxin and vehicle.

3 Thirty-nine pigs were anaesthetized and catheterized for measurement of central and regional haemodynamics. A tonometer in the distal ileum was used for measurement of mucosal PCO_2. Blood gases and plasma ET-1-LI levels as well as histological samples from the gut were assessed. Intervention was started 2 h after onset of endotoxemia and the experiments were terminated after 5 h.

4 Endotoxin-induced changes in systemic, gut oxygen delivery and portal hepatic vascular resistance and systemic acidosis were effectively counteracted by both ET_{A+B}ra and ET_{MIX}ra. ET_Ara administration was not effective while ET_Bra proved to be fatal as all animals in this group died prior to full time of the experiment. While both ET_{A+B}ra and ET_{MIX}ra improved gut oxygen delivery only the latter attenuated the profound endotoxin-induced ileal mucosal acidosis.

5 The lethal effect seen from selective ET_B receptor antagonism in the current study may be due to increased ET_A receptor activity as plasma levels of ET-1 is increased several fold by blocking the ET_B receptor and thereby the plasma-ET-1-clearing function. Furthermore, a loss of endothelial ET_B receptor vasodilating properties may also have contributed to the lethal course in the ET_Bra group

6 The findings in this study suggest that ET is involved in the profound endotoxin-induced disturbances in splanchnic homeostasis in porcine endotoxaemia. Furthermore, antagonism of both ET_A and ET_B receptors is necessary to effectively counteract these changes.

Keywords: Tonometry; septic shock; endotoxin; gut circulation; mucosal damage; endothelin antagonism

Abbreviations: ANOVA, analysis of variance; BE, base excess; CI, cardiac index; CVP, central venous pressure; DMSO, dimethyl sulphoxide; DO_2i, systemic oxygen delivery index; DO_2igut, gut oxygen delivery index; ET, endothelin; ET-1, endothelin-1; ET_A, endothelin A receptor; ET_{A+B}ra, simultaneous adminstration of ET_Ara and ET_Bra; ET_Ara, ET_A antagonist (PD155080); ET-1-LI, endothelin-1-like immunoreactivity; ET_B, endothelin B receptor; ET_Bra, ET_B antagonist (A-192621); ET_{MIX}ra, mixed ET receptor antagonist (A-182086); GutVRI, gut vascular resistance index; Hb, haemoglobin concentration; HR, heart rate; MAP, mean arterial blood pressure; MPOa, myeloperoxidase activity; PCO_2, partial carbon dioxide tension; pHa, arterial pH; pHi, intramucosal pH; PO_2, partial oxygen tension; Portal-hepatic VRI, portal hepatic vascular restistance index; PVP, portal venous pressure; Qpvi, portal venous blood flow index; SaO_2, arterial oxygen saturation; SvO_2, mixed venous oxygen saturation; SVRI, systemic vascular resistance index; VO_2i, systemic oxygen consumption index; VO_2igut, gut oxygen consumption index

Introduction

Septic shock and multiple organ failure remains the leading cause of death in the intensive care unit (Marshall *et al*., 1995). A large number of inflammatory mediators are involved in the complex pathophysiology of sepsis and endotoxaemia. Several of the manifestations such as pulmonary hypertension, regional hypoperfusion and extravasation of leukocytes can be attributed to endothelin (Oldner *et al*., 1998; Wanecek *et al*., 1997). The endothelins are a family of 21 amino acid peptides with powerful vasoconstrictive properties first described in 1988 (Yanagisawa, 1988). Endothelin-1 (ET-1), probably the most important of the endothelins, is mainly produced by the vascular endothelium, acting on three types of receptors. ET_A and ET_{B2} located on vascular smooth muscle cells mediating contraction and ET_{B1} located on the endothelium mediating vasodilation by release of nitric oxide and prostacyclin (Arai *et al*., 1994; Pollock & Opgenorth, 1993; De Nucci *et al*., 1988). Very high plasma levels of ET-1-like immunoreactivity (ET-1-LI) have been demonstrated in various septic conditions and are associated with morbidity and mortality in septic patients (Weitzberg *et al*., 1991a; Pittet *et al*., 1991; Takakuwa *et al*., 1994). A well described feature of septic and endotoxin shock

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is disturbances in splanchnic perfusion and oxygen delivery/demand balance (Dahn *et al*., 1987; Ruokonen *et al*., 1993; Oldner *et al*., 1998). Hypoperfusion of the gut with concomitant mucosal acidosis followed by reduced barrier function and translocation of gut derived bacteria and endotoxin is a proposed aetiology of multiple organ failure in the critically ill patient (Aranow & Fink, 1996). Infusion of ET-1 in humans reduces gut perfusion (Weitzberg *et al*., 1991b). Furthermore, ET-1 reduces liver blood flow in a dose dependent manner and has been advocated as an important meditator of liver perfusion disturbances in septic conditions (Bauer *et al*., 1994; Pannen *et al*., 1996a). In a previous study from our laboratory administration of bosentan (Ro-47-0203, Hoffman La Roche), a non-selective ET-receptor antagonist, during fulminate endotoxin shock in the pig resulted in a total restoration of a markedly reduced gut oxygen delivery and a reversal of endotoxin-induced intestinal mucosal acidosis (Oldner *et al*., 1998). The aim of the present study was to further elucidate the specific role of the ET_A and ET_B receptor, respectively, in gut homeostasis during porcine endotoxaemia. For this purpose selective ET_A and ET_B as well as a mixed ET receptor antagonist were administered separately or in combination during fulminate endotoxin shock in the pig.

Methods

The experimental protocol for this study was approved by the Ethics Committee for experiments in animals, Stockholm, Sweden.

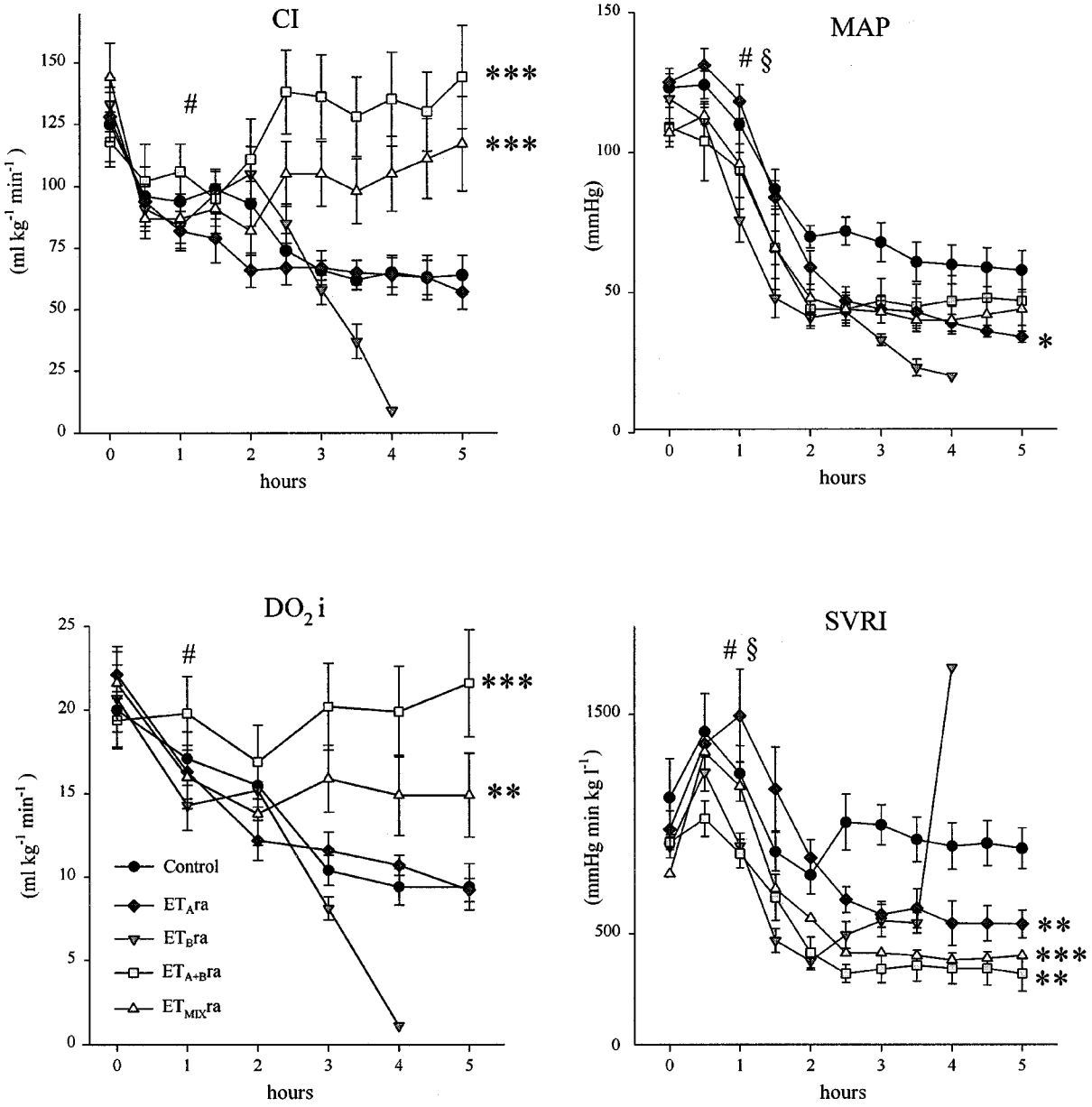

Figure 1 Systemic parameters. Endotoxin control and treated animals. Onset of endotoxin challenge at 0 h and intervention at 2 h. ET_Ara = ET_A receptor antagonist (PD155080); ET_Bra = ET_B receptor antagonist (A-192621); ET_{A+B}ra = simultaneous administration of ET_Ara and ET_Bra; and ET_{MIX}ra = mixed ET receptor antagonist (A-182086). Data presented as mean (s.e.mean). Comparison between groups performed using ANOVA for repeated measures prior to intervention (0–2 h) and post intervention (2.5 or 3–5 h). Significant changes over time prior to intervention for all groups symbolized by #$P<0.01$. Significant differences between control group and treated group prior to intervention symbolized by §$P<0.05$ and during intervention symbolized by *$P<0.05$, **$P<0.01$ and ***$P<0.001$.

Anaesthesia and surgical preparation

Thirty-nine landrace pigs of both sexes, weighing 17.3–23.4 kg, were fasted overnight with free access to water. An intramuscular injection of ketamine 20 mg kg^{-1} and atropine 25 μg kg^{-1} was used for premedication. Anaesthesia was induced by pentobarbital 12 mg kg^{-1} intravenously and maintained by a continuous infusion of pentobarbital 3–6 mg kg^{-1} h^{-1} and fentanyl 5 μg kg^{-1} h^{-1}. Anaesthetic level was evaluated prior to administration of muscle relaxants by pain stimuli to the fore hoof with a forceps. Additional doses of pentobarbital or fentanyl were given when needed. Muscle paralysis was achieved by an intravenous infusion of pancurionum bromide 0.5 mg kg^{-1} h^{-1}. After tracheotomy the animals were mechanically normoventilated with a gas mixture of oxygen in air (fraction of inspiratory O_2 0.30) (Servo 900 ventilator, Siemens Elema, Solna, Sweden). The respiratory frequency was set to 18 resp min^{-1}. Body temperature was maintained at 38–39°C. A balloon tipped pulmonary artery catheter was inserted under pressure guidance *via* a femoral vein to a position in the pulmonary artery. For measurement of arterial blood pressure a catheter was introduced into the abdominal aorta *via* a femoral artery. A continuous infusion of isotonic saline with glucose 2.5 mg ml^{-1} at a rate of 20 ml kg^{-1} h^{-1} was maintained throughout the experiment. A midline laparotomy was performed. A catheter was introduced into the portal vein. An ultrasonic flow probe (Transonic Systems Inc., Ithaca, NY, U.S.A.) for continuous registration of blood flow was placed around the portal vein. For measurement of intestinal mucosal PCO_2 a tonometer (sigmoid catheter, Datex Ohmeda, Helsinki, Finland) was inserted through a small enterotomy in the distal ileum. A catheter was placed in the urinary bladder for collection of urine.

At the end of preparation the abdomen was closed and the animals placed in a left lateral position.

Haemodynamic and blood gas measurements

For continuous measurements and recordings of heart rate (HR) and mean arterial blood pressure (MAP) the arterial catheter was connected to a pressure transducer, while central venous pressure (CVP) and portal venous pressure (PVP) were recorded intermittently on a polygraph (Grass 7B, Quincy, MA, U.S.A.). Cardiac output was measured by thermodilution (Edwards Lab 9520A, St. Ana, CA, U.S.A.) and determined as the mean of a triplicate of 10 ml of ice-cold saline injections and presented as cardiac index (CI, indexed to body weight).

Table 1 Systemic parameters. Endotoxin control and treated animals. Onset of endotoxin challenge at 0 h and intervention at 2 h. Data presented as mean (s.e.mean). Comparison between groups performed using ANOVA for repeated measures prior to intervention (0–2 h) and post intervention (2.5 or 3–5 h)

	0 h	*1 h*	*2 h*	*3 h*	*4 h*	*5 h*
Heart rate (beats min^{-1})						
Control	178 (16)	224 (14)	218 (8)	229 (9)	218 (8)	205 (11)#
ET_Ara	179 (13)	222 (11)	188 (14)	186 (6)	203 (12)	209 (13)#
ET_Bra	165 (17)	234 (11)	195 (13)	199 (8)	125 ()	#
ET_{A+B}ra	135 (14)	232 (10)	196 (14)	223 (12)	226 (16)	213 (24)#
ET_{MIX}ra	143 (8)	198 (17)	193 (13)	224 (10)	220 (16)	222 (18)#
Systemic oxygen consumption index (ml kg^{-1} min^{-1})						
Control	4.0 (0.2)	4.1 (0.2)	4.3 (0.2)	4.6 (0.2)	4.4 (0.2)	4.2 (0.4)
ET_Ara	3.9 (0.5)	3.1 (0.3)	4.1 (0.2)	4.4 (0.4)	4.7 (0.3)	4.4 (0.2)
ET_Bra	4.6 (0.4)	4.3 (0.3)	4.8 (0.2)	3.8 (0.1)	0.7 ()	§
ET_{A+B}ra	4.3 (0.8)	4.5 (0.2)	4.2 (0.4)	4.1 (0.3)	4.5 (0.4)	3.6 (0.8)
ET_{MIX}ra	3.2 (0.3)	3.4 (0.3)	3.7 (0.4)	3.8 (0.5)	3.7 (0.4)	3.2 (0.5)§
Mixed venous oxygen saturation (%)						
Control	77 (3)	73 (3)	68 (3)	50 (5)	46 (5)	47 (6)#
ET_Ara	82 (2)	79 (3)	63 (4)	58 (3)	53 (4)	49 (3)#
ET_Bra	75 (4)	68 (2)	66 (2)	49 (4)	35 ()	#
ET_{A+B}ra	77 (4)	75 (3)	72 (4)	76 (4)	73 (5)	75 (5)#**
ET_{MIX}ra	84 (1)	75 (2)	70 (2)	74 (3)	71 (4)	73 (5)#**
Arterial pH						
Control	7.40 (0.02)	7.32 (0.02)	7.24 (0.03)	7.16 (0.03)	7.13 (0.04)	7.15 (0.04)#
ET_Ara	7.40 (0.01)	7.35 (0.01)	7.22 (0.03)	7.14 (0.03)	7.15 (0.02)	7.15 (0.03)#
ET_Bra	7.39 (0.03)	7.34 (0.01)	7.24 (0.02)	7.16 (0.01)	7.18 ()	#
ET_{A+B}ra	7.47 (0.03)	7.36 (0.03)	7.28 (0.04)	7.29 (0.04)	7.27 (0.04)	7.28 (0.04)#*
ET_{MIX}ra	7.43 (0.01)	7.33 (0.02)	7.29 (0.02)	7.25 (0.02)	7.27 (0.02)	7.29 (0.03)#*
Base excess (mM)						
Control	−2.5 (1.0)	−6.5 (1.7)	−9.8 (1.8)	−13.4 (1.7)	−15.0 (1.9)	−13.8 (2.0)#
ET_Ara	2.3 (0.7)	−2.8 (1.0)	−11.5 (1.5)	−13.3 (1.8)	−14.0 (1.1)	−15.4 (1.5)#
ET_Bra	−1.9 (1.2)	−6.4 (1.3)	−8.8 (1.3)	−14.3 (0.08)	−19.4 ()	#
ET_{A+B}ra	0.8 (1.2)	−3.2 (2.0)	−7.1 (2.0)	−6.6 (2.1)	−8.2 (2.0)	−7.7 (2.3)#*
ET_{MIX}ra	0.1 (0.5)	−4.5 (0.8)	−7.4 (1.1)	−8.4 (1.3)	−8.3 (1.6)	−8.4 (1.9)#*
Haemoglobin concentration (g l^{-1})						
Control	117 (4)	137 (4)	125 (3)	118 (5)	112 (6)	113 (8)#
ET_Ara	127 (5)	148 (4)	138 (5)	132 (6)	131 (9)	126 (9)#§
ET_Bra	112 (4)	125 (4)	107 (6)	107 (3)	95 ()	#§
ET_{A+B}ra	119 (5)	136 (4)	114 (7)	110 (5)	110 (7)	110 (7)#
ET_{MIX}ra	108 (3)	135 (5)	121 (8)	111 (6)	106 (5)	103 (6)#

Significant changes over time prior to intervention symbolized by #$P<0.001$. Significant differences between control group and treated group prior to intervention symbolized by §$P<0.05$ and during intervention symbolized by *$P<0.05$. **$P<0.01$, ***$P<0.001$.

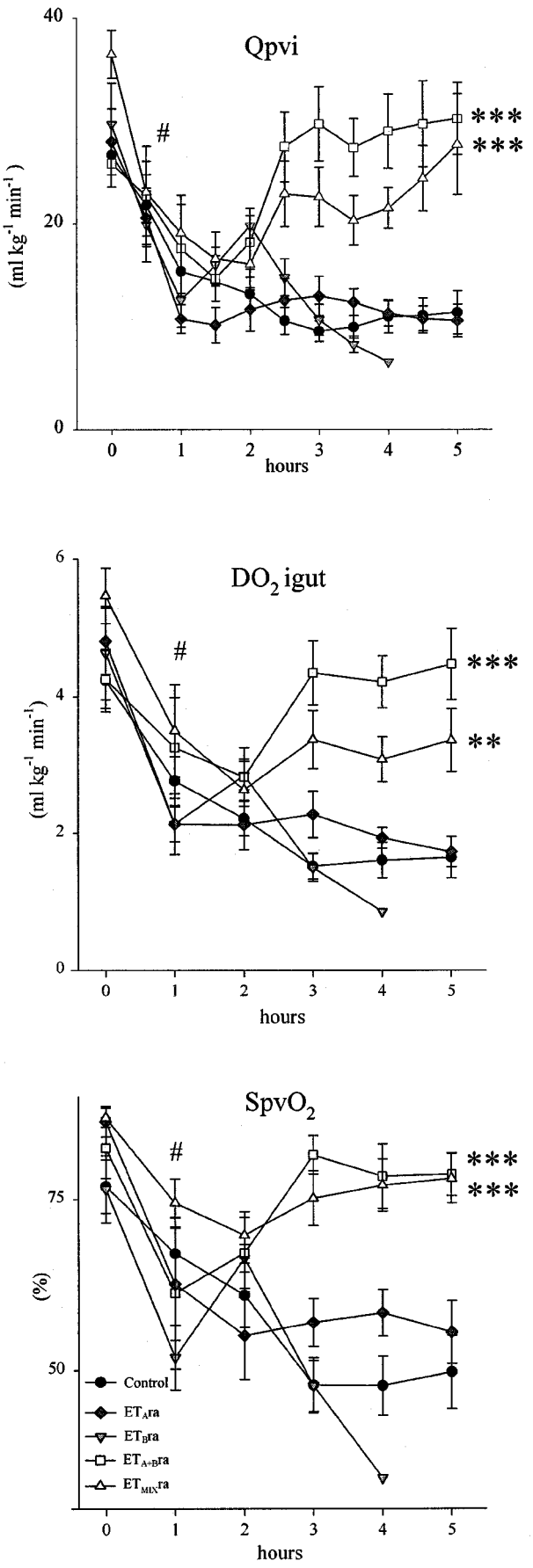

Figure 2 Splanchnic parameters. Endotoxin control and treated animals. Onset of endotoxin challenge at 0 h and intervention at 2 h. ET_Ara = ET_A receptor antagonist (PD155080); ET_Bra = ET_B antagonist receptor (A-192621); ET_{A+B}ra = simultaneous administration of ET_Ara and ET_Bra; and ET_{MIX}ra = mixed ET receptor antagonist (A-182086). Data presented as mean (s.e.mean). Comparison between groups performed using ANOVA for repeated measures prior to intervention (0–2 h) and post intervention (2.5 or 3–5 h). Significant changes over time prior to intervention for all groups symbolized by #P < 0.01. Significant differences between control group and treated group during intervention symbolized by $^{**}P < 0.01$, $^{***}P < 0.001$.

Systemic vascular resistance index (SVRI) was calculated as: [(MAP − CVP) Cl^{-1}]. Portal blood flow was recorded continuously on the polygraph and indexed to body weight (Qpvi), presented as ml min^{-1} kg^{-1}. Gut vascular resistance index (GutVRI, including pancreas and spleen) was calculated as: [(MAP − PVP) $Qpvi^{-1}$]. The portal venous hepatic vascular resistance index (portal-hepatic VRI) was calculated as: [(PVP − CVP) $Qpvi^{-1}$]. Blood was collected from the arterial, pulmonary artery and portal venous catheters for analysis of blood gases and acid base status (PO_2, PCO_2, pH, HCO_3^- and base excess (BE)) on an ILS 1610 blood gas analyzer (Instrumentation laboratories, Warrington, Cheshire, U.K.). Systemic oxygen delivery index (DO_2i) was calculated as: [$SaO_2 \times Hb \times 0.0139 \times CI$] and systemic oxygen consumption index (VO_2i) as: [SaO_2 − mixed venous oxygen saturation (SvO_2) × Hb × 0.0139 × CI]. Gut oxygen delivery index (DO_2i-gut, including pancreas and spleen) was calculated as: [Qpvi × Hb × 0.0139 × SaO_2] and gut oxygen consumption index (VO_2igut, including pancreas and spleen) as: [Qpvi × Hb × 0.0139 × SaO_2-portal venous oxygen saturation].

Biochemical analysis

Arterial and portal plasma levels of endothelin-1-like immunoreactivity (ET-1-LI) were analysed with radioimmunoassay as described by Hemsén (Hemsén, 1991). Hb was measured spectrophotometrically (Haemoglobin photometer, LEO, Helsingborg, Sweden).

Endotoxin

Escherichia coli lipopolysaccharide endotoxin (serotype 0111:B4, Sigma, St. Louis, U.S.A.), dissolved in a saline and warmed in order to dissolve any precipitate was used.

Tissue analysis

Biopsies were taken from the distal ileum prior to endotoxaemia ($n = 8$) and in the control ($n = 6$), ET_{A+B}ra ($n = 5$) and ET_{MIX}ra ($n = 4$) groups at termination of the experiment. Biopsies were placed on a Millipore filter, fixed in 4% neutral formalin, imbedded in paraffin, sectioned at 4 μ and stained with hematoxilin and eosin. The material was blinded to a pathologist and the histomorphological changes graded in accordance to Chiu *et al.* (1970). Ileal biopsies were also taken at termination of the experiment from the control ($n = 5$), ET_Ara ($n = 5$), ET_{A+B}ra ($n = 5$) and ET_{MIX}ra ($n = 4$) groups and immediately frozen to −80°C for analysis of myeloperoxidase activity (MPOa). Full-thickness tissue was homogenized and the MPO activity was measured as described previously (Schierwagen *et al.*, 1990).

Experimental protocol

After surgical preparation the animals were allowed 1 h stabilization. An intravenous endotoxin infusion was started at 0 h at a rate of 2.5 μg kg^{-1} h^{-1} and increased stepwise during 30 min to reach a final infusion rate of 20 μg kg^{-1} h^{-1}.

The endotoxin infusion was discontinued after 3 h and the animals were observed for another 2 h. After 2 h of endotoxin infusion, eight animals received an intravenous bolus injection of the ET_A-receptor antagonist (ET_Ara) PD155080 (Park Davis, Ann Arbor, MI, U.S.A.) of 10 mg kg^{-1} dissolved in isotonic saline followed by a continuous infusion of 5 mg kg^{-1} h^{-1} for the rest of the experiment. Seven animals received a bolus injection of the ET_B-receptor antagonist (ET_Bra) A-192621 (Abbott Laboratories, Chicago, IL, U.S.A.) of 10 mg kg^{-1} dissolved in 2% dimethyl sulphoxide (DMSO) followed by a continuous infusion of 5 mg kg^{-1} h^{-1} for the rest of the experiment. Six animals received a combination of ET_Ara and ET_Bra (ET_{A+B}ra) administered as in the groups above. Eight animals received an intravenous bolus injection of the mixed ET-receptor antagonist (ET_{MIX}ra) A-182086 (Abbott Laboratories, IL, U.S.A.) of 5 mg kg^{-1} dissolved in distilled (deionized) water followed by a continuous infusion of 0.5 mg kg^{-1} h^{-1} for the rest of the experiment. Ten animals receiving only endotoxin served as control group. From these controls six received the DMSO vehicle at 2 h while four received a saline vehicle. MAP, HR and portal venous blood flow were monitored continuously. Every 30 min CO was measured, CVP and PVP were recorded and CI, SVRI, GutVRI and portal-hepatic VRI were calculated. Blood samples were collected from the aorta, pulmonary artery and portal vein catheters for analysis of blood gases, in addition Hb and arterial plasma levels of ET-1-LI were analysed hourly. PCO_2 in the saline obtained from the tonometer measured every hour was used for calculation of intramucosal mucosal pH (pHi) by means of Henderson-Hasselbalch's equation ($pHi = 6.1 + \log [HCO_3^-]/[PCO_{2(SS)} \times 0.03]$ where HCO_3^- is the bicarbonate level in arterial blood and $PCO_{2(SS)}$ is the PCO_2 level at steady state in the saline from the tonometer). Since the saline in the balloon is not fully equilibrated with the luminal PCO_2 after 1 h, a correction table, provided by the manufacturer, was used to obtain PCO_2 at steady state ($PCO_{2(SS)}$). Mucosal-arterial PCO_2gap was calculated as: [$PCO_{2(SS)}$ − arterial PCO_2]. Mucosal-portal PCO_2gap was calculated as [$PCO_{2(SS)}$ − portal venous PCO_2].

At 5 h the experiments were terminated and the animals were sacrificed by a lethal dose of pentobarbital injected into a central vein.

Statistics

Data are presented as mean (±s.e.mean). An univariate analysis for repeated measures of variance (ANOVA) was used for comparison between groups and changes over time from 0–2 h (prior to intervention) and 2.5 (or 3)–5 h (during intervention) respectively. The time point 2 h was used as covariate for the ANOVA during intervention. All treatment groups were compared to the control group. No statistical analysis was made between the treatment groups. Control animals receiving DMSO vehicle ($n=6$) were compared to controls receiving saline vehicle ($n=4$) using ANOVA as described above. Kruskal-Wallis test, if significant, followed by Mann Whitney *U*-test was used for comparison between control group and other groups for histological grading and MPOa data obtained from biopsies. Differences were considered significant at $P<0.05$. A computer software

Table 2 Splanchnic parameters. Endotoxin control and treated animals. Onset of endotoxin challenge at 0 h and intervention at 2 h. Data presented as mean (s.e.mean). Comparison between groups performed using ANOVA for repeated measures prior to intervention (0–2 h) and post intervention (2.5 or 3–5 h)

	0 h	*1 h*	*2 h*	*3 h*	*4 h*	*5 h*
Portal blood flow index cardiac index^{-1} *ratio* (%)						
Control	22 (2)	16 (2)	14 (1)	14 (1)	16 (1)	17 (2)#
ET_Ara	22 (2)	13 (1)	17 (2)	19 (2)	18 (1)	19 (1)#
ET_Bra	22 (2)	15 (3)	20 (2)	19 (4)	75 ()	#
ET_{A+B}ra	22 (1)	16 (3)	17 (2)	23 (2)	23 (3)	22 (3)#
ET_{MIX}ra	26 (2)	23 (4)	22 (4)	24 (4)	24 (3)	26 (3)#
Gut vascular resistance index (mmHg min kg l^{-1})						
Control	4.8 (0.7)	7.9 (1.4)	5.7 (1.4)	6.4 (1.3)	5.7 (1.7)	5.3 (1.3)#
ET_Ara	4.5 (0.6)	11.3 (2.0)	5.0 (1.2)	2.3 (0.4)	2.3 (1.0)	2.1 (0.3)#
ET_Bra	4.1 (0.5)	6.1 (0.9)	1.7 (0.3)	2.2 (0.3)	1.4 ()	#
ET_{A+B}ra	3.9 (0.9)	5.4 (0.8)	2.1 (0.5)	1.4 (0.4)	1.4 (0.4)	1.3 (0.4)#
ET_{MIX}ra	2.5 (0.1)	5.2 (1.0)	2.6 (0.4)	1.4 (0.1)	1.3 (0.2)	1.4 (0.3)#
Portal venous pressure (mmHg)						
Control	10 (0.9)	13 (0.9)	11 (0.9)	13 (0.81)	12 (0.6)	12 (0.8)#
ET_Ara	11 (0.9)	14 (0.9)	14 (1.4)	15 (0.8)	15 (1.0)	15 (0.8)#
ET_Bra	6 (1.2)	10 (2.7)	9 (2.1)	11 (2.7)	11 ()	#
ET_{A+B}ra	9 (1.6)	10 (1.7)	9 (1.3)	8 (1.2)	10 (1.1)	9 (1.3)# **
ET_{MIX}ra	7 (1.5)	13 (1.4)	12 (1.4)	11 (1.6)	13 (1.1)	11 (1.2)#
Gut oxygen consumption index (ml kg^{-1} min^{-1})						
Control	0.74 (0.08)	0.70 (0.09)	0.69 (0.07)	0.71 (0.07)	0.69 (0.07)	0.70 (0.08)
ET_Ara	0.56 (0.09)	0.67 (0.15)	0.77 (0.07)	0.85 (0.09)	0.75 (0.08)	0.68 (0.04)
ET_Bra	0.96 (0.12)	0.94 (0.15)	0.97 (0.08)	0.72 (0.04)	0.56 ()	§
ET_{A+B}ra	0.68 (0.17)	0.87 (0.24)	0.85 (0.13)	0.69 (0.13)	0.76 (0.12)	0.82 (0.10)
ET_{MIX}ra§	0.68 (0.06)	1.03 (0.28)	0.86 (0.13)	0.95 (0.20)	0.82 (0.15)	0.80 (0.10)§
Portal-hepatic vascular resistance index (mmHg min^{-1} kg^{-1})						
Control	0.30 (0.08)	0.73 (0.16)	0.85 (0.33)	1.23 (0.17)	1.09 (0.27)	1.20 (0.33)#
ET_Ara	0.22 (0.04)	0.89 (0.23)	1.08 (0.25)	0.94 (0.18)	0.89 (0.12)	0.91 (0.13)#
ET_Bra	0.21 (0.03)	1.04 (0.40)	0.40 (0.06)	1.07 (0.20)	0.91 ()	#
ET_{A+B}ra	0.29 (0.06)	0.67 (0.19)	0.43 (0.10)	0.23 (0.04)	0.29 (0.05)	0.25 (0.05)#*
ET_{MIX}ra	0.12 (0.01)	0.55 (0.17)	0.54 (0.12)	0.33 (0.07)	0.40 (0.08)	0.29 (0.06)#*

Significant changes over time prior to intervention symbolized by #$P<0.05$. Significant differences between control group and treated group prior to intervention symbolized by §$P<0.05$ and during intervention symbolized by *$P<0.05$, **$P<0.01$.

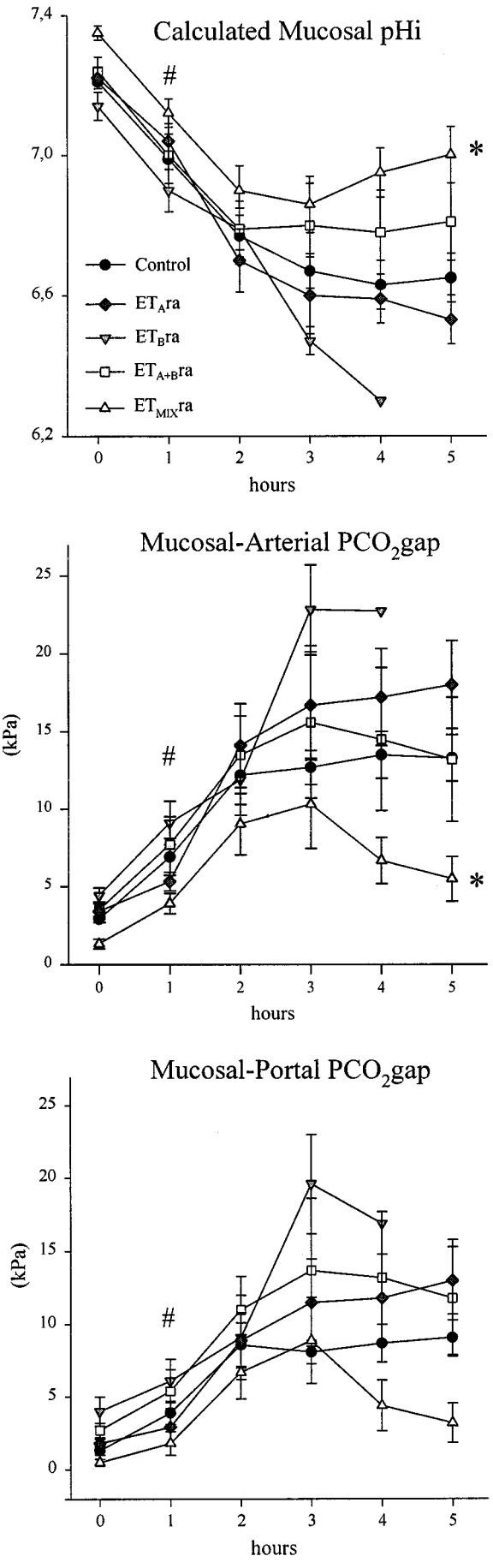

Figure 3 Tonometrical parameters. Endotoxin control and treated animals. Onset of endotoxin challenge at 0 h and intervention at 2 h. ET_Ara = ET_A receptor antagonist (PD155080); ET_Bra = ET_B receptor antagonist (A-192621); ET_{A+B}ra = simultaneous administration of ET_Ara and ET_Bra; and ET_{MIX}ra = mixed ET receptor antagonist (A-182086). Data presented as mean (s.e.mean). Comparison between groups performed using ANOVA for repeated measures prior to intervention (0–2 h) and post intervention (3–5 h). Significant changes over time prior to intervention for all groups symbolized by #P<0.05. Significant differences between control group and treated group during intervention symbolized by *$P<0.05$.

program (Statistica 5.1, StatSoft Inc., Tulsa, OK, U.S.A.) was utilized for statistical calculations.

Results

All animals subject to ET_Bra treatment died from progressive hypodynamic shock prior to termination of experiments. For this reason there were no statistical calculations made between the ET_Bra animals and the control group during the intervention period. The effect of ET_Bra treatment is not further commented under results. In addition, one animal in the ET_Ara group died prior to termination while all other animals survived for full time (5 h) of the experiments.

Systemic haemodynamics, oxygen delivery and consumption (Figure 1 and Table 1)

Endotoxaemia resulted in notable decrease in CI and DO_2i that was counteracted by ET_{A+B}ra and ET_{MIX}ra treatment while ET_Ara administration had no effect on these parameters. Endotoxin infusion increased HR significantly while no intervention further affected this parameter. MAP was reduced in response to endotoxin infusion. A significant difference was noted between the control group and the ET_Bra and ET_{MIX}ra group prior to onset of intervention at 2 h. MAP was slightly but significantly reduced by ET_Ara treatment while no differences were seen between the ET_{A+B}ra and ET_{MIX}ra groups and control animals. After an initial peak at aproximately 0.5 h SVRI was mainly unchanged in the control group. A significant difference between ET_Bra and controls was seen prior to intervention at 2 h. ET_Ara, ET_{A+B}ra and ET_{MIX}ra treatment all reduced SVRI significantly as compared to control animals. VO_2i differed significantly between the ET_Bra, ET_{MIX}ra group and control animals prior to onset of intervention. No differences were observed between groups in response to intervention. In accordance with the changes in DO_2i the SvO_2 was significantly reduced during endotoxaemia. ET_{A+B}ra and ET_{MIX}ra treatment resulted in a reversal of this reduction while the decrease was further accentuated in the other groups.

Regional haemodynamics, oxygen delivery and consumption (Figure 2 and Table 2)

Both Qpvi and DO_2igut were profoundly reduced by endotoxin challenge. Moreover, these reductions were more pronounced than on the systemic level as the Qpvi Cl^{-1} ratio was significantly reduced during the initial phase of endotoxaemia. Both ET_{A+B}ra and ET_{MIX}ra treatment effectively counteracted the endotoxaemia-induced reduction in Qpvi and DO_2igut. ET_Ara treatment alone had no effect on these parameters. A tendency by ET_{A+B}ra treatment to increase the Qpvi Cl^{-1} ratio compared to controls did not reach statistical significance ($P<0.053$). GutVRI increased substantially in response to endotoxaemia with an initial peak at approxi-

mately 1 h. A non-significant tendency to reduction was seen in response to ET_Ara ($P<0.07$), ET_{A+B}ra ($P<0.08$) and ET_{MIX}ra ($P<0.07$) treatment. Endotoxin infusion induced an increase in PVP and only ET_{A+B}ra treatment reduced this parameter significantly. A significant difference was seen in VO_2igut between ET_Bra and control animals prior to intervention while no significant differences were observed between groups during intervention. Endotoxin infusion reduced portal venous oxygen saturation markedly. Treatment with ET_{A+B}ra and ET_{MIX}ra reversed this reduction effectively. No improvements were seen in the other treatment groups as compared to controls. Portal-hepatic VRI increased notably in response to endotoxin administration. This increase was effectively counteracted by both the ET_{A+B}ra combination and ET_{MIX}ra treatment while no changes were seen by ET_Ara treatment alone.

Intestinal tonometry (Figure 3)

Endotoxin challenge induced profound changes in parameters obtained by tonometry. Intestinal mucosal pHi was notably reduced while increases were seen in both mucosal-arterial PCO_2gap and mucosal-portal PCO_2gap. These data demonstrate mucosal susceptibility to endotoxin. Despite the clear increase in DO_2igut in response to both ET_{A+B}ra and ET_{MIX}ra treatment only the latter was able to improve pHi and reduce mucosal-arterial PCO_2gap. None of the treatments improved mucosal-portal PCO_2gap.

Acid-base status and haemoglobin (Table 1)

Both ET_{A+B}ra and ET_{MIX}ra treatment reversed the endotoxin-induced metabolic acidosis seen as reductions in BE and pHa. No beneficial effects were seen from the other treatments. Haemoconcentration with a peak at approximately 1 h was noted during the initial phase of endotoxaemia. Significant differences were seen between the ET_Ara, ET_Bra and control group prior to intervention while no significant differences were seen thereafter.

Intestinal histology and myeloperoxidase activity (Figures 4 and 5)

Endotoxemia induced clear histological damage to the ileal mucosa where control animals had a significantly higher grade as compared to biopsies obtained prior to endotoxaemia ($P<0.003$). A tendency for both ET_{A+B}ra and ET_{MIX}ra treatment to reduce these histological changes did not reach statistical significance ($P<0.09$ and 0.12 respectively). No significant differences between groups were observed for MPOa in the ileal biospsies.

Endothelin-1-like immunoreactivity (Figure 6)

Endotoxin induced a progressive increase in plasma ET-1-LI in control animals and at 5 h the levels were 4 fold higher than at baseline. The ET-1-LI levels were profoundly increased by administration of ET_B receptor antagonists as administration of ET_Bra, ET_{A+B}ra and ET_{MIX}ra at 2 h generated a 10 fold increase compared to baseline levels, significantly higher than the control group. No further increase in plasma ET-1-LI was seen upon ET_Ara administration.

Saline and DMSO vehicle

No significant differences were observed between control animals receiving saline ($n=4$) and DMSO ($n=6$) vehicle respectively in any parameter except for Qpvi which was slightly lower in DMSO vehicle animals (data not shown).

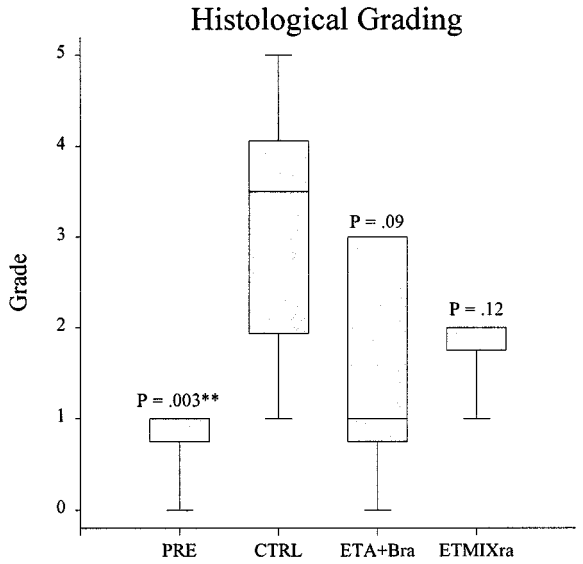

Figure 4 Box plot of histological intestinal mucosal damage. Grading from 0–5 according to Chiu *et al.* (1970). Biopsies obtained prior to endotoxaemia (PRE, $n=8$) first box, control animals (CTRL) at 5 h ($n=6$) second box, ET_{A+B}ra (simultaneous administration of the ET_A receptor antagonist (PD155080) and the ET_B receptor antagonist (A-192621)) treated animals at 5 h ($n=5$) third box and ET_{MIX}ra (mixed ET receptor antagonist (A-182086)) treated animals at 5 h ($n=4$) fourth box. Biopsies obtained prior to endotoxaemia and from treatment groups compared to control animals by Kruskal-Wallis test if significant followed by Mann-Whitney *U*-test. *P* values displayed in figure. Data presented as median (line), 25–75% (box), min–max (error bars).

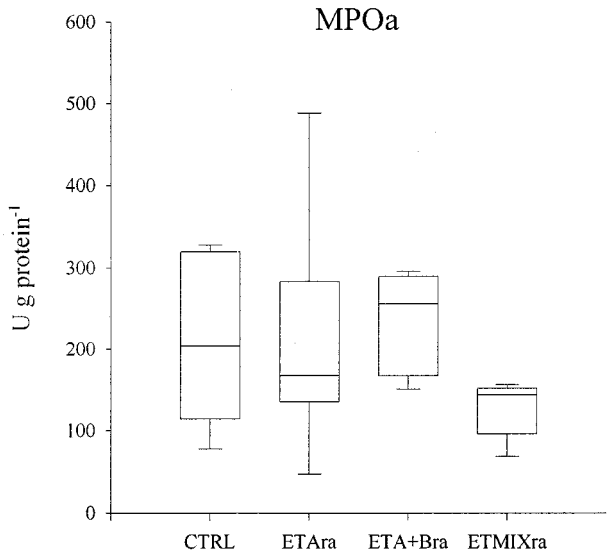

Figure 5 Box plot of ileal myeloperoxidase activity. Displayed as units per gram protein content. Biopsies obtained from control animals at 5 h ($n=5$) first box, ET_Ara (ET_A receptor antagonist (PD155080)) treated animals ($n=5$) at 5 h second box, ET_{A+B}ra (simultaneous administration of the ET_A receptor antagonist (PD155080) and the ET_B receptor antagonist (A-192621)) treated animals ($n=6$) at 5 h third box and ET_{MIX}ra (mixed ET receptor antagonist (A-182086) treated animals at 5 h ($n=4$) fourth box. Kruskal-Wallis test for group comparison was not significant. Data presented as median (line), 25–75% (box), min–max (error bars).

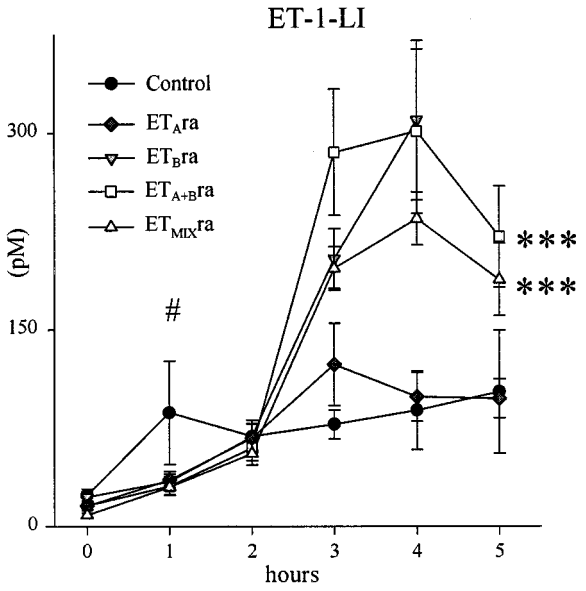

Figure 6 Endothelin-1 like immunoreactivity in arterial plasma. Endotoxin control and treated animals. Onset of endotoxin challenge at 0 h and intervention at 2 h. Data presented as mean (s.e.mean). Comparison between groups performed using ANOVA for repeated measures prior to intervention (0–2 h) and post intervention (3–5 h). Significant changes over time prior to intervention for all groups symbolized by #$P<0.05$. Significant differences between control group and treated group during intervention symbolized by ***$P<0.001$.

Discussion

A major finding in this study is that simultaneous administration of selective ET_A (PD155080) and ET_B (A-192621) as well as a non-selective endothelin receptor antagonist (A-182086) significantly increased both systemic and gut oxygen delivery and reversed systemic acidosis. Furthermore, the non-selective ET receptor antagonist reduced ileal mucosal acidosis. In contrast, separate administration of the ET_A receptor antagonist provided no major beneficial effects while detrimental effects were noted from selective ET_B receptor antagonism alone.

Under physiological conditions ET-1 is constantly produced and participates in regulation of vascular tone (Weitzberg *et al*., 1994). It acts mostly as a paracrine mediator mainly secreted abluminally suggesting that plasma levels reflect only a fraction of the total activity. However, increased production may be seen in various conditions such as in septic and endotoxin shock (Weitzberg *et al*., 1991a; Pittet *et al*., 1991; Takakuwa *et al*., 1994). During endotoxaemia the increased synthesis and release of endothelin results in several fold increases in plasma levels and ET-1 may then act as a circulating mediator (Ahlborg *et al*., 1995). Infusion of ET-1 in healthy volunteers induces some of the manifestations seen in sepsis, such as, reduced splanchnic and renal blood flows, lowered cardiac output and pulmonary hypertension (Weitzberg *et al*., 1991b; 1993). Apart from perfusion disturbances several other features of sepsis and endotoxin shock may be related to endothelin receptor activity. Activation of leukocytes with subsequent extravasation and production of myeloperoxidase and cytokines as well as renal dysfunction have all been demonstrated in response to ET (Lopez Farre *et al*., 1993; Helset *et al*., 1994; Rubanyi & Polokoff, 1994; Cunningham, 1997).

In this study CI decreased early in the course of endotoxaemia while MAP decreased somewhat later generating a notable transient increase in SVRI. Administration of ET_Ara did not affect CI but reduced MAP and SVRI suggesting presence of ET_A receptor-mediated vasoconstriction in response to endotoxaemia. Both ET_{MIX}ra and ET_{A+B}ra restored CI and reduced SVRI without affecting MAP suggesting that addition of ET_B receptor antagonism is important for cardiac output in this model of endotoxaemia. Paradoxically, all animals that received the ET_B receptor antagonist alone died prior to full time of the experiment. One important function of the ET_B receptor is clearance of plasma ET (Dupuis *et al*., 1996). Antagonism of the ET_B receptor increases circulating ET-1 levels several fold in particular during endotoxaemia (Dupuis *et al*., 1996; Oldner *et al*., 1998). Thus, the subsequent increase in ET_A receptor stimulation as a consequence of the elevated ET-1 levels may have contributed to the lethal effect of ET_B receptor antagonism seen in this study. In addition, loss of endothelial ET_{B1} receptor-mediated vasodilation modulating the ET_A receptor-induced vasoconstriction may also have contributed to the lethal response to selective ET_B receptor antagonism. A direct toxic effect by ET_Bra is not likely as administration simultaneously with ET_Ara proved to be beneficial. The subtle effects seen from ET_Ara treatment suggest that ET_B receptor activity is of pathophysiological importance in the current model but that the beneficial effects from ET_Bra treatment may be concealed unless the ET_A receptor is antagonized simultaneously. The changes and treatment-response in DO_2i did not differ from CI. Surprisingly, despite the marked initial decrease and total restoration of DO_2i in two of the treatment groups no corresponding changes were observed in VO_2i suggesting a lack of oxygen supply dependency in this model.

Administration of endotoxin notably reduced both Qpvi and DO_2igut. This reduction was more pronounced in the gut than in general as the Qpvi Cl^{-1} ratio decreased significantly during early endotoxin shock. A reduction in gut oxygen delivery is not a mandatory finding during sepsis and endotoxaemia. Increases in gut oxygen delivery often accompanied by even more pronounced increases in gut oxygen consumption are well-described findings in human septic shock (Dahn *et al*., 1987; Ruokonen *et al*., 1993). In experimental settings the response is very much dependent on the model utilized. Species, way of shock induction and amount of volume loading may all affect the response. In the current model administration of a non-selective ET-receptor antagonist or simultaneous administration of the selective ET_A and ET_B receptor antagonists effectively counteracted the endotoxin-induced reduction in Qpvi and DO_2igut. In contrast, use of a selective ET_A receptor antagonist did not affect these parameters and selective ET_B receptor antagonism proved to be fatal. A tendency for ET_Ara, ET_{A+B}ra and ET_{MIX}ra to reduce GutVRI did not reach statistical significance. The finding that non-selective antagonism of ET receptors is effective is consistent with previous studies including a study from our laboratory where, bosentan, a non-selective ET receptor antagonist completely reversed the profound reduction in gut oxygen delivery in a similar experimental model (Wilson *et al*., 1993; Oldner *et al*., 1998). The lack of beneficial effects by selective ET_A receptor administration, except for a tendency to reduce GutVRI, is somewhat surprising as this receptor has been advocated as an important mediator of splanchnic perfusion disturbances (Massberg *et al*., 1998; Kayashima *et al*., 1998). Miura *et al*. (1996) could demonstrate increased red blood cell velocity and reduced mucosal damage in the ileum when pretreating endotoxaemic rats with BQ123 a selective ET_A receptor antagonist. Massberg *et al*. (1998) effectively counteracted

ET-1 induced intestinal mucosal damage in rats pretreated with ET_A receptor antagonists while ET_B receptor antagonism proved to be non effective.

Mucosal acidosis as seen in the present study is a common feature in septic and endotoxin shock and may have important clinical bearings. Translocation of gut derived endotoxin and bacteria across a hyperpermeable gut barrier have been suggested to promote multiple organ failure in critically ill patients (Deitch, 1990). Reductions in mucosal pHi have in several clinical studies been associated with bad outcome in this group of patients (Doglio *et al*., 1991; Gutierrez *et al*., 1992). The use of mucosalarterial PCO_2gap, i.e. the difference in carbon dioxide tension between the mucosa as obtained by tonometry and arterial blood has been advocated as a more precise measure of mucosal oxygenation than calculated pHi. Respiratory acid-base changes and infusion of buffering agents may affect calculated pHi without reflecting primary changes in the mucosal oxygenation (Benjamin *et al*., 1992a,b). In the present study both calculated mucosal pHi and mucosal-arterial PCO_2gap deteriorated markedly in response to endotoxaemia. This was also the case for mucosal-portal PCO_2gap, utilized as a measure of the intestinal mucosa in relation to the gut in general (including the spleen and pancreas). The changes seen reflect mucosal vulnerability during endotoxaemia. Administration of ET antagonists at 2 h had differentiated effects on tonometrical parameters. Only treatment with ET_{MIX}ra was able to improve calculated pHi and mucosal-arterial PCO_2gap while a tendency by this drug to reduce mucosal-portal PCO_2gap did not reach statistical significance. Surprisingly, the combination of ET_{A+B}ra did not improve any of these parameters despite a total restoration of gut oxygen delivery in this group. No difference between ET_{A+B}ra and ET_{MIX}ra was seen in the ileal biopsies where both treatments had a tendency, not reaching statistical significance, to improve histological grading that was markedly affected by endotoxaemia. Even though biopsies were obtained from a limited number of animals there was a discrepancy to tonometrical parameters making interpretation of the mucosal effects by ET_{A+B}ra and ET_{MIX}ra somewhat difficult. However, in a previous study from our laboratory, administration of bosentan also restored gut oxygen delivery and reduced mucosal acidosis. These differences in findings may be due to different effects of these drugs on the intestinal microcirculation. The balance between ET_A and ET_B receptor antagonism may be of importance for these effects. A dominant ET_A receptor blockade profile has been shown *in vitro* for bosentan (approximately 20 : 1) and A-182086 (approximately 4 : 1), the drugs with the most beneficial effects on mucosal pHi in the current study (Clozel, 1994). The relation between gut oxygen delivery and mucosal homeostasis is complex in sepsis and endotoxaemia. Increases in regional oxygen consumption and microcirculatory dysregulation may contribute to acidosis in these conditions (Ruokonen *et al*., 1993; Dahn *et al*., 1987; Takala, 1997; Humer *et al*., 1996; Schumacker, 1996). Alterations in blood flow distribution between mucosa and muscularis may generate mucosal acidosis without apparent changes in regional blood flow (Schumacker, 1996). Moreover, disturbances in mitochondrial respiration reducing oxygen utilization capacity during endotoxaemia and sepsis have been suggested to contribute to mucosal acidosis even in the presence of oxygen (Fink, 1997; Unno *et al*., 1997). ET may also exercise harmful effects on mucosal integrity during non-endotoxaemic conditions. In haemorrhagic shock ET antagonism may protect against shock-induced gastric mucosal ulcerations (Michida *et al*., 1994; Kitajima *et al*., 1995) while local administration of ET may induce mucosal damage in non-shocked animals (Whittle & Lopez-Belmonte, 1993; Lopez-Belmonte & Whittle, 1994). Increases in MPOa following activation of neutrophils may be seen in inflammatory states and have been demonstrated in response to ET-1 infusion and in experimental colitis pretreatment with bosentan reduced colonic MPOa in rats (Lopez Farre *et al*., 1993; Hogaboam *et al*., 1996). In the current study no reduction of MPOa was seen in response to treatment, findings consistent with a study by Alican *et al*. where no effect was seen in response to ET antagonism in a model of gut ischaemia-reperfusion in the rat (Alican *et al*., 1998).

The blood flow through the gut is also dependent on the outflow conditions through the liver (Ayuse *et al*., 1995). Endotoxaemia-induced increases in PVP, as seen in the current study, may contribute to splanchnic blood pooling and edema formation. Interestingly, regulation of liver perfusion seems to depend largely on ET-receptor activity during endotoxaemia. Infusion of ET-1 into the portal vein markedly reduces sinusoidal blood flow in a dose-dependent manner (Bauer *et al*., 1994). Release of ET-1 from sinusoidal endothelial cells acting on Ito cells mediating contraction has been one suggested flow regulating mechanism (Tanikawa, 1995). Moreover, endotoxin has been reported to enhance portal venous contractile response to ET-1. Pannen *et al*. showed that bosentan only slightly reduced hepatic vascular resistance in sham animals while it had a notable effect during endotoxaemia (Pannen *et al*., 1996b). In the current study endotoxaemia induced a 6 fold increase in portal-hepatic VRI in the control group. Non-selective ET-receptor antagonism effectively counteracted the endotoxaemia-induced increase in this parameter reaching close to baseline levels at 5 h. These findings support the concept of ET being highly important in liver blood flow regulation during endotoxaemia. Both ET_A and ET_B receptors have been reported to mediate vasoconstrictive response in the liver (Zhang *et al*., 1995) but the reports in the literature concerning the relative importance of these receptors are not uniform. Partly in agreement with the findings in the present study Iwai *et al*. noted that hepatocellular damage and blood flow disturbances in perfused rat livers in response to ET-1 infusion were aggravated by both selective ET_Ara and ET_Bra while simultaneous administration resulted in improvement of both parameters (Iwai *et al*., 1998). Likewise Zhang *et al*. (1997) demonstrated that combined ET_A and ET_B antagonism was necessary to fully antagonize the effect of ET in isolated perfused rat livers. However, both ET_A and ET_B receptor antagonism were partly effective in that study. In contrast, Ruetten *et al*. observed reduced hepatocellular injury in endotoxaemic rats treated with ET_B but not ET_A receptor antagonists (Ruetten & Thiemermann, 1996) and Nishida *et al*. (1998) noted an aggravated endotoxin-induced hepatic injury in rats treated with selective ET_A receptor antagonists. Thus, the effects of ET on the liver are complex and heterogeneous. Portal-hepatic VRI as calculated in the current study is an estimate of the portal portion of hepatic vascular resistance. Under physiological conditions reductions in portal blood flow is compensated by a decrease in hepatic arterial resistance, an adenosine-dependent mechanism referred to as the hepatic arterial buffer response (Lautt *et al*., 1985). The changes in portal blood flow and increase in portal-hepatic VRI in the present study may be of particular importance if not compensated for by hepatic arterial blood flow. In endotoxaemia the buffer response has been demonstrated to be impaired (Ayuse *et al*., 1995). In a recent study by Bathe *et al*. (1998) the hepatic arterial blood flow was more reduced than portal venous blood flow in endotoxaemic pigs. These

data suggest that the changes seen in current study may not have been compensated for by an increase in hepatic arterial blood flow.

A central feature in shock is cellular energy depletion with ATP hydrolysis generating hydrogen ions (Gutierrez & Wulf, 1996). In the current study a profound endotoxin-induced metabolic acidosis was counteracted by both ET_{A+B}ra and ET_{MIX}ra. The increase in both systemic and regional oxygen delivery seen in these groups is likely to have contributed to this finding.

The vehicle used for ET_Bra in the present study, DMSO, may exert anti-oxidant properties. Wray *et al*. (1998) recently reported reduced liver injury in endotoxaemic rats treated with DMSO. However, no significant differences were seen between DMSO and non-DMSO treated control animals with the exception of Qpvi that was slightly lower in the DMSO group. These data suggest that DMSO did not exert any favourable effects in the present study.

The relative pathophysiological importance of ET_A as opposed to ET_B receptor activity in sepsis and endotoxaemia is complex. The literature is inconsistent and the findings vary substantially with the experimental model in terms of species, way of shock induction and duration of experiment. Moreover, the results from pretreating as opposed to treatment during fulminate shock as in the current study may differ substantially. The situation may be further complicated by interaction between receptors referred to as receptor 'cross-talk'. Ozaki *et al*. (1997) demonstrated reduced ET_B receptor affinity for both ET_B receptor agonists and antagonists when ET_A receptors in the same cell was stimulated. Therefore, data obtained in one model regarding relative importance of subgroups of ET receptors may not prove valid in an other. Clearly, the role of specific ET receptors in human septic pathophysiology must be further elucidated.

In conclusion, in this porcine model administration of endotoxin resulted in profound reductions in both systemic and gut oxygen delivery as well as a notable intestinal mucosal acidosis. Administration of a selective ET_A receptor antagonist was not followed by any clear beneficial effects while administration of a selective ET_B receptor antagonist proved to be fatal as all animals in this group succumbed prior to termination of the experiment. No animals died in the control group. In contrast, simultaneous administration of the two selective antagonists markedly improved both systemic and gut oxygen delivery and systemic metabolic acidosis as well as reduced portal-hepatic vascular resistance but not mucosal acidosis while administration of the non-selective ET receptor antagonist also reversed mucosal acidosis. The lethal effect seen from selective ET_B receptor antagonism alone in the current study may be due to increased ET_A receptor activity and concomitant blockade of ET_{B1} receptor-mediated vasodilation, as plasma levels of ET-1 is increased several fold by blocking the plasma-ET-1-clearing function of the ET_B receptor.

The findings in this study suggest that ET is strongly involved in the profound disturbances in splanchnic homeostasis in procine endotoxaemia and that simultaneous antagonism of both ET_A and ET_B receptors are necessary to counteract these changes.

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References

AHLBORG, G., WIETZBERG, E. & LUNDBERG, J.M. (1995). Circulating endothelin-1 reduces splanchnic and renal blood flow and splanchnic glucose production in humans. *J. Appl. Physiol.,* **79,** 141 – 145.

ALICAN, I., YEGEN, C., OLCAY, A., KURTEL, H. & YEGEN, B. (1998). Ischemia-reperfusion-induced delay in intestinal transit. Role of endothelins. *Digestion,* **59,** 343 – 348.

ARAI, H., HORI, S., ARAMORI, I., OHKUBO, H. & NAKANISHI, S. (1994). Cloning and expression of a cDNA encoding an endothelin receptor. *Nature,* **348,** 730 – 732.

ARANOW, J.S. & FINK, M.P. (1996). Determinants of intestinal barrier failure in critical illness. *Br. J. Anaesth.,* **77,** 71 – 81.

AYUSE, T., BRIENZA, N., REVELLY, J.P., ODONNELL, C.P., BOITNOTT, J.K. & ROBOTHAM, J.L. (1995). Alterations in liver hemodynamics in an intact porcine model of endotoxin shock. *Am. J. Physiol. Heart Circ. Phy.,* **37,** H1106 – H1114.

BATHE, O.F., RUDSTON-BROWN, B., CHOW, A.W.C. & PHANG, P. (1998). Liver as a focus of impaired oxgygenation and cytokine production in a porcine model of endotoxicosis. *Crit. Care Med.,* **26,** 1698 – 1706.

BAUER, M., ZHANG, J.X., BAUER, I. & CLEMENS, M.G. (1994). ET-1 induced alterations of hepatic microcirculation: sinusoidal and extrasinusoidal sites of action. *Am. J. Physiol.,* **267,** G143 – G149.

BENJAMIN, E., HANNON, E.M., OROPELLO, J.M., STERN, P.M., PREMUS, G. & IBERTI, T.J. (1992a). Effects of respiratory acidosis on gastrointestinal tonometry. *Anesthesiology,* **77,** A307.

BENJAMIN, E., POLOKOFF, E., OROPELLO, J.M., LEIBOWITZ, A.B. & IBERTI, T.J. (1992b). Sodium bicarbonate administration affects the diagnostic accuracy of gastrointestinal tonometry in acute mesenteric ischemia. *Crit. Care Med.,* **20,** 1181 – 1183.

CHIU, C.J., MCARDLE, A.H., BROWN, R., SCOTT, H.J. & GURD, F.N. (1970). Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. *Arch. Surg.,* **101,** 478 – 483.

CLOZEL, M. (1994). Pharmacological characterization of bosentan, a new potent orally active non-peptide endothelin receptor antagonist. *J. Pharmacol. Exp. Ther.,* **270,** 228 – 235.

CUNNINGHAM, M. (1997). Endothelin-1 and endothelin-4 stimulate monocyte production of cytokines. *Crit. Care Med.,* **25,** 958 – 964.

DAHN, M.S., LANGE, P., LOBDELL, K., HANS, B., JACOBS, L.A. & MITCHELL, R.A. (1987). Splanchnic and total body oxygen consumption differences in septic and injured patients. *Surgery,* **101,** 69 – 80.

DEITCH, E.A. (1990). The role of intestinal barrier failure and bacterial translocation in the development of systemic infection and multiple organ failure. *Arch. Surg.,* **125,** 403 – 404.

DE NUCCI, G., THOMAS, R., D'ORLEANS-JUSTE, P., ANTUNES, E., WALDER, C., WARNER, T.D. & VANE, J.R. (1988). Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc. Natl. Acad. Sci. U.S.A.,* **85,** 9797 – 9800.

DOGLIO, G.R., PUSAJO, J.F., EGURROLA, M.A., BONFIGLI, G.C., PARRA, C., VETERE, L., HERNANDEZ, M.S., FERNANDEZ, S., PALIZAS, F. & GUTIERREZ, G. (1991). Gastric mucosal pH as a prognostic index of mortality in critically ill patients. *Crit. Care Med.,* **19,** 1037 – 1040.

DUPUIS, J., GORESKY, C. & FOURNIER, A. (1996). Pulmonary clearance of circulating endothelin-1 in dogs in vivo: exclusive role of ETB receptors. *J. Appl. Physiol.,* **81,** 1510 – 1515.

FINK, M. (1997). Cytopathic hypoxia in sepsis. *Acta Anaestesiol. Scand.,* **41,** 87 – 95.

GUTIERREZ, G., PALIZAS, F., DOGLIO, G., WAINSZTEIN, N., GALLESIO, A., PACIN, J., DUBIN, A., SCHIAVI, E., JORGE, M., PUSAJO, J, KLEIN, F., SAN ROMAN, E., DORFMAN, B., SHOTTLENDER, J. & GINIGER, R. (1992). Gastric intramucosal pH as a therapeutic index of tissue oxygenation in critically ill patients. *Lancet,* **339,** 195 – 199.

GUTIERREZ, G. & WULF, M.E. (1996). Lactic acidosis in sepsis: a commentary. *Int. Care Med.,* **22,** 6 – 16.

HELSET, E., YTREHUS, K., TVEITA, T., KJAEVE, J. & JORGENSEN, L. (1994). ET-1 causes accumulation of leukocytes in the pulmonary circulation. *Circ. Shock,* **44,** 201 – 209.

HEMSÉN, A. (1991). Biochemical and functional characterization of endothelin peptides with special references to vascular effects. *Acta Physiol. Scand.,* **142,** 5 – 61.

HOGABOAM, C.M., MULLER, M.J., COLLINS, S.M. & HUNT, R.H. (1996). An orally active non-selective endothelin receptor antagonist, bosentan, markedly reduces injury in a rat model of colitis. *Eur. J. Pharmacol.,* **309,** 261 – 269.

HUMER, M.F., PHANG, P.T., FRIESEN, B.P., ALLARD, M.F., GODDARD, C.M. & WALLEY, K.R. (1996). Heterogeneity of gut capillary transit times and impaired gut oxygen extraction in endotoxemic pigs. *J. Appl. Physiol.,* **81,** 895 – 904.

IWAI, M., YAMAUCHI, T. & SHIMAZU, T. (1998). Endothelin 1 aggravates acute liver injury in perfused livers of rats after treatment with D-galactosamine. *Hepatology,* **28,** 503 – 509.

KAYASHIMA, K., KUDO, H., DOI, Y. & FUJIMOTO, S. (1998). The role of endothelin-1 in regulation of rat mesenteric microcirculation. *J. Cardiovasc. Pharmacol.,* **31,** S126 – S127.

KITAJIMA, T., TANI, K., YAMAGUCHI, T., KUBOTA, Y., OKUHIRA, M., MIZUNO, T. & INOUE, K. (1995). Role of endogenous endothelin in gastric mucosal injury induced by hemorrhagic shock in rats. *Digestion,* **56,** 111 – 116.

LAUTT, W.W., LEGARE, D.J. & D'ALMEIDA, M.S. (1985). Adenosine as putative regulator of hepatic arterial flow (the buffer response). *Am. J. Physiol.,* **248,** H331 – H338.

LOPEZ-BELMONTE, J. & WHITTLE, B.J. (1994). The involvement of endothelial dysfunction, nitric oxide and prostanoids in the rat gastric microcirculatory responses to endothelin-1. *Br. J. Pharmacol.,* **112,** 267 – 271.

LOPEZ FARRE, A., RIESCO, A., ESPINOSA, G., DIGIUNI, E., CERNADAS, M.R., ALVAREZ, V., MONTON, M., RIVAS, F., GALLEGO, M.J., EGIDO, J., CASADO, S. & CARAMELO, C. (1993). Effect of endothelin-1 on neutrophil adhesion to endothelial cells and perfused heart. *Circulation,* **88,** 1166 – 1171.

MARSHALL, J., COOK, D., CHRISTOU, N.V., BERNARD, G.R., SPRUNG, C.L. & SIBBALD, W.J. (1995). Multiple organ dysfunction score: a reliable descriptor of a complex clinical outcome. *Crit. Care Med.,* **23,** 1638 – 1652.

MASSBERG, S., BOROS, M., LEIDERER, R., BARANYI, L., OKADA, H. & MESSMER, K. (1998). Endothelin (ET)-1 induced mucosal damage in the rat small intestine: role of ET_A receptors. *Shock,* **9,** 177 – 183.

MICHIDA, T., KAWANO, S., MASUDA, E., KOBAYASHI, I., NISHIMURA, Y., TSUJII, M., HAYASHI, N., TAKEI, Y., TSUJI, S., NAGANO, K. , FASAMOTO, H. & KAMADA, T. (1994). Role of endothelin 1 in hemorrhagic shock-induced gastric mucosal injury in rats. *Gastroenterology,* **106,** 988 – 993.

MIURA, S., FUKUMURA, D., KUROSE, I., HIGUCHI, H., KIMURA, H., TSUZUKI, Y., SHIGEMATSU, T., HAN, J.Y., TSUCHIYA, M. & ISHII, H. (1996). Roles of ET-1 in endotoxin-induced microcirculatory disturbance in rat small intestine. *Am. J. Physiol.,* **271,** G461 – G469.

NISHIDA, T., HUANG, T.P., SEIYAMA, A., HAMADA, E., KAMIIKE, W., UESHIMA, S., KAZUO, H. & MATSUDA, H. (1998). Endothelin A-receptor blockade worsens endotoxin-induced hepatic microcirculatory changes and necrosis. *Gastroenterology,* **115,** 412 – 420.

OLDNER, A., WANECEK, M., GOINY, M., WEITZBERG, E., RUDEHILL, A., ALVING, K. & SOLLEVI, A. (1998). The endothelin receptor antagonist bosentan restores gut oxygen delivery and reverses intestinal mucosal acidosis in porcine endotoxin shock. *Gut,* **42,** 696 – 702.

OZAKI, S., OHWAKI, K., IHARA, M., ISHIKAWA, K., YANO, M. (1997). Coexpression studies with endothelin receptor subtypes indicate the existence of intracellular cross-talk between ET_A and ET_B receptors. *J. Biochem.,* **121,** 440 – 447.

PANNEN, B.H., BAUER, M., ZHANG, J.X., ROBOTHAM, J.L. & CLEMENS, M.G. (1996a). Endotoxin pretreatment enhances portal venous contractile response to endothelin-1. *Am. J. Physiol.,* **270,** H7 – H15.

PANNEN, B.H., BAUER, M., ZHANG, J.X., ROBOTHAM, J.L. & CLEMENS, M.G. (1996b). A time-dependent balance between endothelins and nitric oxide regulating portal resistance after endotoxin. *Am. J. Physiol.,* **271,** H1953 – H1961.

PITTET, J.F., MOREL, D.R., HEMSEN, A., GUNNING, K., LACROIX, J.S., SUTER, P.M. & LUNDBERG, J.M. (1991). Elevated plasma endothelin-1 concentrations are associated with the severity of illness in patients with sepsis. *Ann. Surg.,* **213,** 261 – 264.

POLLOCK, D.M. & OPGENORTH, T.J. (1993). Evidence for endothelin-induced renal vasoconstriction independent of endothelinA receptor activation. *Am. J. Physiol.,* **264,** R222.

RUBANYI, G.M. & POLOKOFF, M.A. (1994). Endothelins: molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. *Pharmacol. Rev.,* **46,** 325 – 415.

RUETTEN, H. & THIEMERMANN, C. (1996). Effect of selective blockade of endothelin ET_B receptors on the liver dysfunction and injury caused by endotoxaemia in the rat. *Br. J. Pharmacol.,* **119,** 479 – 486.

RUOKONEN, E., TAKALA, J., KARI, A., SAXEN, H., MERTSOLA, J. & HANSEN, E.J. (1993). Regional blood flow and oxygen transport in septic shock. *Crit. Care Med.,* **21,** 1296 – 1303.

SCHIERWAGEN, C., BYLUND-FELLENIUS, A.-C. & LUNDBERG, C. (1990). Improved method for quantification of tissue PMN accumulation measured by myeloperoxidase activity. *J. Pharmacol. Meth.,* **23,** 179 – 186.

SCHUMACKER, P. (1996). Regulation of gut oxygen delivery, cellular oxygen supply and metabolic activity. In: Rombeau, J.L., Takala, J. (eds). *Gut dysfunction in critical illness*. Vol. 26, Springer: Berlin, pp 65 – 75.

TAKAKUWA, T., ENDO, S., NAKAE, H., KIKICHI, M., SUZUKI, T., INADA, K. & YOSHIDA, M. (1994). Plasma levels of TNF-alpha, endothelin-1 and thrombomodulin in patients with sepsis. *Res. Comm. Chem. Pathol. Pharmacol.,* **84,** 261 – 269.

TAKALA, J. (1997). Regional contribution to hypermetabolism following trauma. *Baillieres Clin. Endocrinol. Metab.,* **11,** 617 – 627.

TANIKAWA, K. (1995). Hepatic sinusoidal cells and sinusoidal circulation. *J. Gastroenterol. Hepatol.,* **10,** S8 – S11.

UNNO, N., WANG, H., MENCONI, M.J., TYGTAT, S.H.A.J., LARKIN, V., SMITH, M., MORIN, M.J., CHAVEZ, A., HODIN, R.A. & FINK, M.P. (1997). Inhibition of inducible nitric oxide synthase ameliorates endotoxin-induced gut mucosal barrier dysfunction in rats. *Gastroenterology,* **113,** 1246 – 1257.

WANECEK, M., OLDNER, A., RUDEHILL, A., SOLLEVI, A., ALVING, K. & WEITZBERG, E. (1997). Cardiopulmonary dysfunction during porcine endotoxin shock is effectively counteracted by the endothelin receptor antagonist bosentan. *Shock,* **7,** 364 – 370.

WEITZBERG, E., AHLBORG, G. & LUNDBERG, J.M. (1991b). Long-lasting vasoconstriction and efficient regional extraction of endothelin-1 in human splanchnic and renal tissues. *Biochem. Biophys. Res. Comm.,* **180,** 1298 – 1303.

WEITZBERG, E., AHLBORG, G. & LUNDBERG, J.M. (1993). Differences in vascular effects and removal of endothelin-1 in human lung, brain, and skeletal muscle. *Clin. Physiol.,* **13,** 653 – 662.

WEITZBERG, E., LUNDBERG, J.M. & RUDEHILL, A. (1991a). Elevated plasma levels of endothelin in patients with sepsis syndrome. *Circ. Shock.,* **33,** 222 – 227.

WEITZBERG, E., RUDEHILL, A., MODIN, A. & LUNDBERG, J.M. (1994). Porcine intrinsic pulmonary and systemic vascular tone is endothelin-dependent. *Acta Physiol. Scand.,* **152,** 433 – 434.

WHITTLE, B.J. & LOPEZ-BELMONTE, J. (1993). Actions and interactions of endothelins, prostacyclin and nitric oxide in the gastric mucosa. *J. Physiol. Pharmacol.,* **44,** 91 – 107.

WILSON, M.A., STEEB, G.D. & GARRISON, R.N. (1993). Endothelins mediate intestinal hypoperfusion during bacteremia. *J. Surg. Res.,* **55,** 168 – 175.

WRAY, G.M., HINDS, C.J. & THIEMERMANN, C. (1998). Effects of inhibitors of poly (ADP-ribose) synthase activity on hypotension and multiple organ dysfunction caused by endotoxin. *Shock,* **10,** 13–19.

YANAGISAWA, M. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature,* **332,** 411–415.

ZHANG, B., CALMUS, Y., WEN, L., SOGNI, P., LOTERSZTAJN, S., HOUSSIN, D. & WEILL, B. (1997). Endothelin-1 induces liver vasoconstriction through both ET_A and ET_B receptors. *J. Hepatol.,* **26,** 1104–1110.

ZHANG, J.X., BAUER, M. & CLEMENS, M.G. (1995). Vessel and target cell specific actions of endothelin-1 and endothelin-3 in rat liver. *Am. J. Physiol.,* **269,** G269–G277.

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